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Coprophagic relations among the earthworms *Eisenia foetida*, *Eudrilus eugeniae* and *Amynthas* spp.[1])

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With 9 figures

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1. Introduction

As increasing amounts of sludge from wastewater treatment plants are diverted away from natural waterways and into landscapes as a result of public law 92–500 (Anonymous 1976), which was passed in 1972, it becomes correspondingly important to determine the rate at which organic wastes can be mineralized in soil. Since earthworms often comprise the largest fraction of animal biomass in certain soils (Murphy 1953; Barley 1961), and are significant in aerating and mixing soil components (Satchell 1958), a potential exists for use of these organisms in accelerating the process of mineralization.

Dindal et al. (1978) observed a significant increase in earthworm biomass in soil receiving secondary effluents of a wastewater treatment plant, while Curry (1976) reported toxic effects upon earthworms when excessive cattle slurry is placed upon soil. A series of experiments in our laboratory (Hartenstein 1978a), along with publications by Graff (1974), Watanabe & Tsukamoto (1976), and Hartenstein (1978b), suggest the earthworm *Eisenia foetida* (Savigny) may be used in management of organic wastes and production of protein (Schulz & Graff 1977; Sabine 1978).

Other earthworm species may function similarly (Hartenstein 1978b). *Eudrilus eugeniae* (Kinberg), for example, requires a high percentage of organic matter in its diet and grows about twice as fast as *E. foetida* on certain sludges (unpublished observations). Since *E. foetida* and *E. eugeniae* can be produced commercially in large quantity, and have a potential for use in waste management, we examined them with respect to coprophagy. We also examined a mixture of two pheretemoid species, since they feed predominantly on mineral soil and tolerate high temperatures (Watanabe 1975). These studies were initiated in view of a preliminary observation that the sludge castings of *E. foetida* are toxic to this earthworm (Hartenstein 1978a).

2. Materials and methods

An activated sludge from the Meadowbrook-Limestone Treatment Plant, Onondaga County, NY, was obtained from drying beds. Mineral topsoil, a Teel silt loam, was purchased locally. *E. foetida*, *E. eugeniae*, and the pheretemoid species were obtained from stock cultures in our laboratory. The pheretemoids were identified by Dr. E. G. Easton as *Amynthas hawayanus* (Rosa, 1891) and *A. rodericensis* (Grube, 1879).

Enough *E. foetida*, *E. eugeniae* and *Amynthas* spp. were used to convert a large quantity of soil or sludge to castings within two weeks. Samples of the castings and sludge not used in the initial run were allowed to age before being assayed. Thirty grams of soil or soil castings, and 40 g of sludge or sludge castings, were placed in 100×20 mm petri dishes for assay. Soil, 1 cm deep, covered the bottom of the plates where indicated in certain experiments with sludge castings. Each dish contained an individual worm which was weighed initially and at 3 and 6 days. A minimum of four

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replicates were run for each treatment. Only immature *E. foetida* and *E. eugeniae* were used, since their rapid growth rates would facilitate measurements of effect of test-substrates on the species. Only adult *Amynthas* spp. were available, and thus used, for testing.

Determination of whether toxicity of castings was due to a water soluble or insoluble substance was tested by assay of fresh sludge following addition of water extracts from castings. Extractions with water at room temperature were carried out with 1/1 (v/w) distilled water to sludge castings of about 70% moisture in flasks shaken for 1 h on an orbital shaker at 75 rpm. After centrifuging in a Sorvall SS3 with a GSA rotor at 5000 rpm for 10 min, the supernatant was used to saturate an uningested sample of sludge which was then shaken and centrifuged as above. Soil and soil castings were aged and tested at 0, 2 and 4 weeks. Sludge and sludge castings were aged and tested at 0 and 6 weeks. In a separate experiment, sludge and sludge castings were aged and tested at 0, 1, 3 and 4 weeks.

Determination of whether toxicity of castings from *E. foetida* is attributable to microbial pathogens was tested by bioassay of the worms in autoclaved (120 °C, 15 psi, 30 min)[1]) castings or sludge and in castings or sludge irradiated with 4000 rads/h from ^{60}Co for 1 week. Bioassay was carried out by placing earthworms for 2 weeks into about 200 cc of control and treated sludge and castings in 4.5 cm high × 10.5 cm dia. glass culture dishes. Twenty and 15 worms were used per dish in the experiments involving autoclaving and irradiation respectively.

Oxidation-reduction potential, Eh, were recorded following a 10 min contact between the electrodes and test materials, using a Corning Model 130 digital *p*H meter. All experimental procedures including aging of soils and sludges, were done at 25 °C.

3. Results

3.1. Soil castings

Values of *p*H and Eh of the soil castings are given in Table 1. All materials had a high redox potential, in excess of 250 mv, and a *p*H between 6.0 and 7.2.

Figure 1 illustrates the toxicity of fresh soil castings from *E. foetida* to itself and the other earthworm species; neither the castings of *E. eugeniae* nor those of *Amynthas* spp. were toxic to themselves, to one another or to *E. foetida*. All of the *E. eugeniae* and *E. foetida* were killed within 6 days upon being restricted to the soil castings of *E. foetida* as a substrate, while the *Amynthas* spp. lost 50% of their weight and number. Though not shown in Figure 1, all of the worms maintained or gained weight when restricted to previously unconsumed soil.

Table 1. Redox potential (millivolts) and *p*H of soil and castings of soil

	Time (weeks)					
	0		2		4	
	*p*H	mv	*p*H	mv	*p*H	mv
Soil	6.7	+440	6.6	+362	7.2	+293
Soil castings, *Eisenia*	6.0	+484	6.4	+386	7.1	+387
Soil castings, *Eudrilus*	6.6	+448	6.5	+427	6.7	+395
Soil castings, *Amynthas*	6.4	+483	6.4	+457	6.6	+450

Table 2. Redox potential (millivolts) and *p*H of sludge and castings of sludge

	Time (weeks)			
	0		6	
	*p*H	mv	*p*H	mv
Activated sludge	6.8	+256	5.5	+419
Sludge castings, *Eisenia*	4.8	+400	4.7	+450
Sludge castings, *Eudrilus*	4.8	+389	5.3	+437
Sludge castings, *Amynthas*	5.0	+387	5.3	+451

[1]) 1 psi = 1 lbf/in² = 6.89476 kPa (kPa = Kilo-Pascal); 1 la = 1 N/m² = 1 m^{-1} · kg · s^{-2}

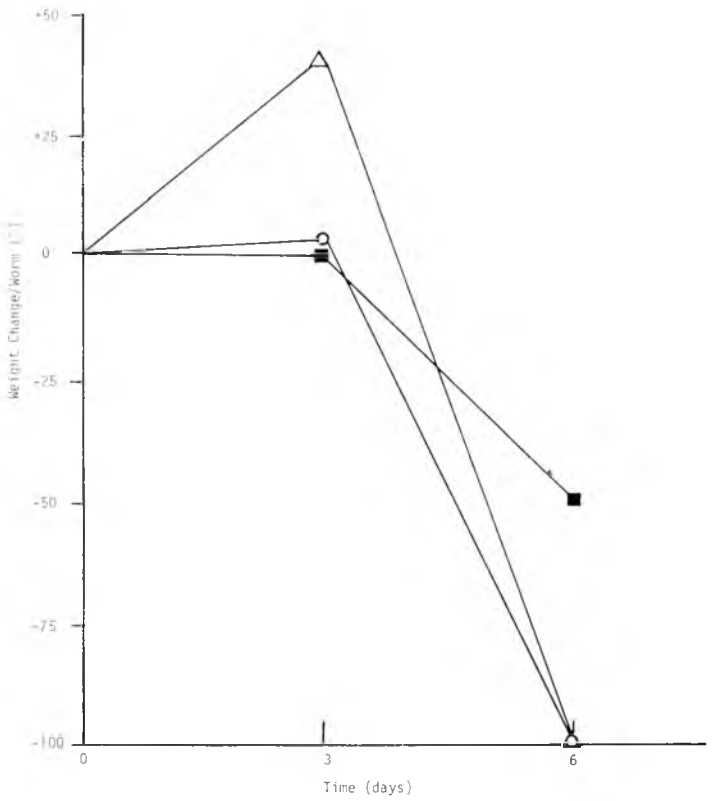

Fig. 1. Effect of soil castings from *E. foetida* on weight of *E. foetida* (○), *Amynthas* spp. (■) and *E. eugeniae* (△).

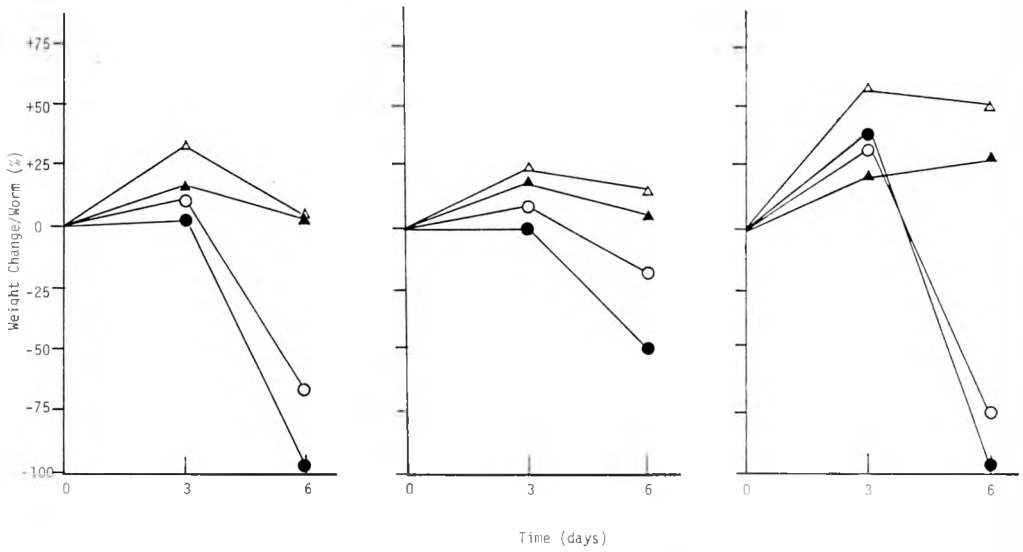

Fig. 2. Effect of *E. foetida* soil castings (●), water-extracted *E. foetida* soil castings (○), soil amended with water extracts from castings of *E. foetida* (▲), and soil (△) on *E. foetida* (left), *Amynthas* spp. (center) and *E. eugeniae* (right).

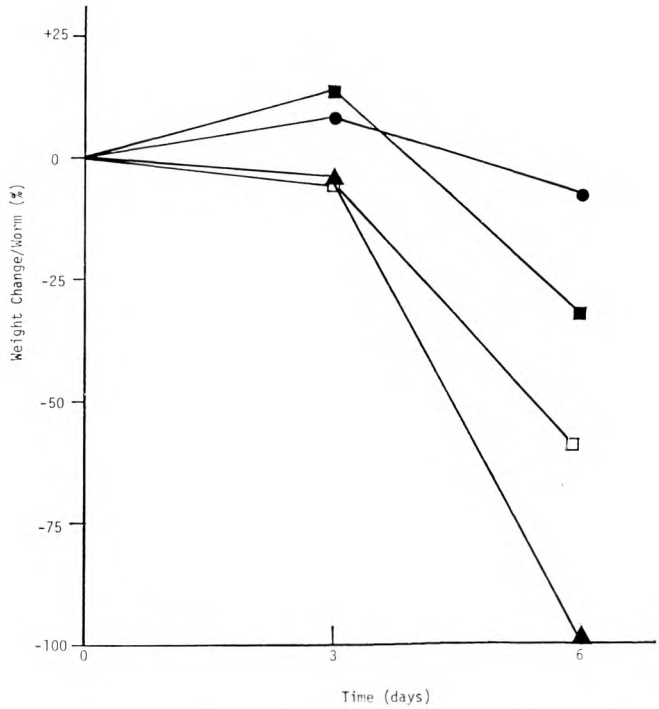

Fig. 3. Effect of soil (○), fresh soil castings from *E. foetida* (▲), castings aged 2 weeks (□), and 4 weeks (□) on weight of *E. foetida*.

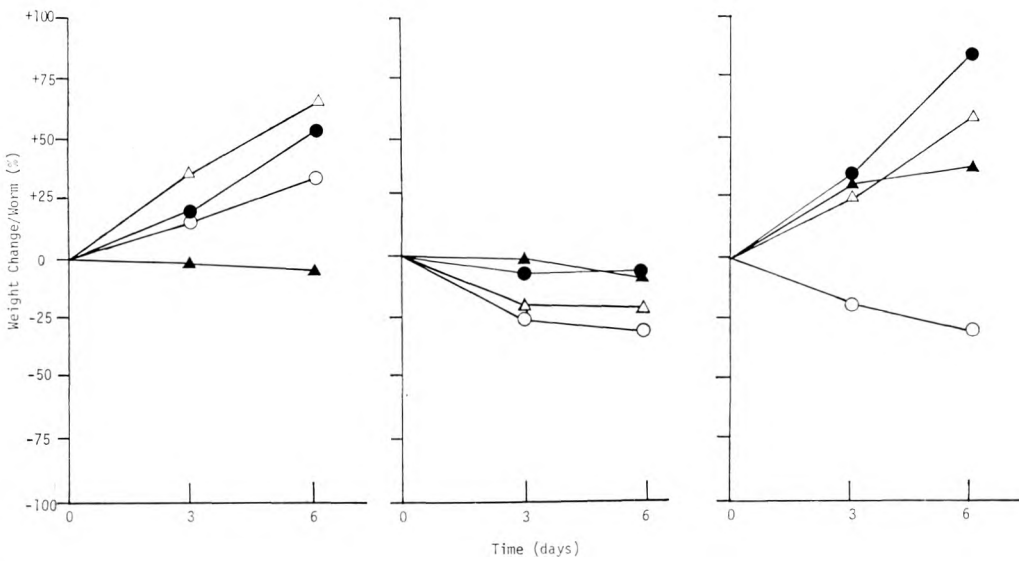

Fig. 4. Effect of sludge (●), and fresh sludge castings from *E. eugeniae* (○), *Amynthas* spp. (△) and *E. foetida* (▲) on weight of *E. foetida* (left), *E. eugeniae* (center), and *Amynthas* spp. (right).

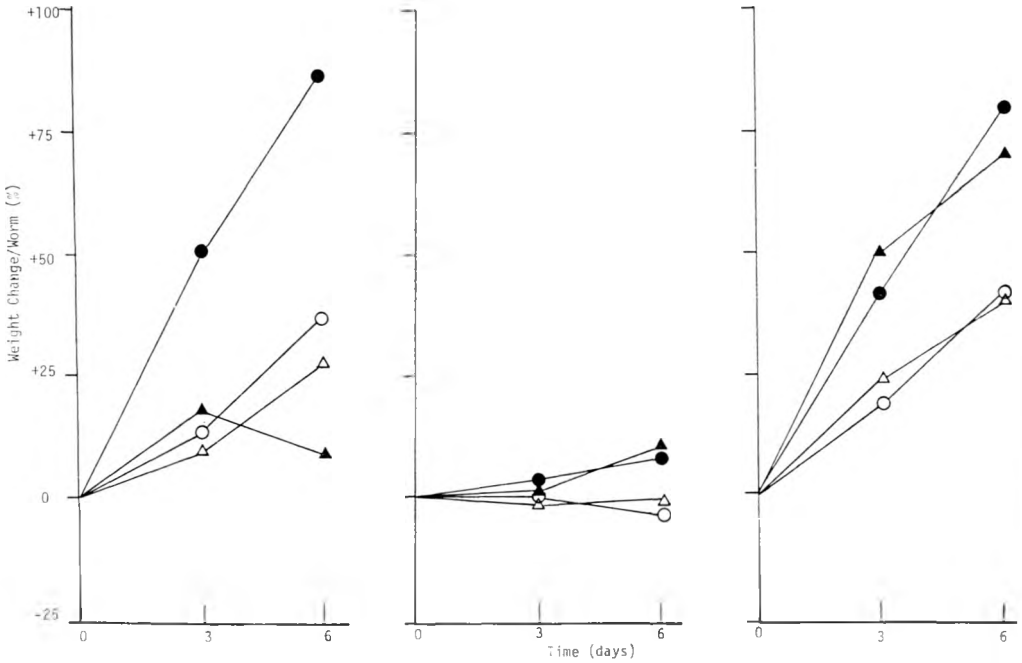

Fig. 5. Effect of sludge (●), and sludge castings from *E. eugeniae* (○), *E. foetida* (▲), and *Amyntha* spp. (△) on weight of *E. foetida* (left), *Amynthas* spp. (center), and *E. eugeniae* (right). In contrast to experiments presented in Fig. 4, the castings had been placed upon soil.

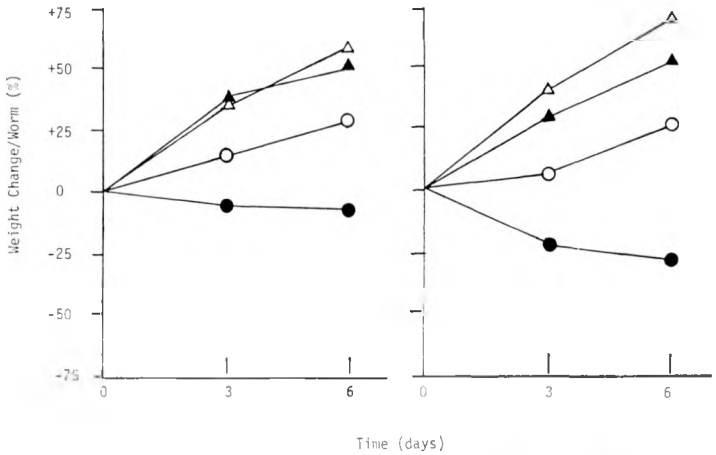

Fig. 6. Intraspecific effect of sludge castings (●), water-extracted sludge castings (○), water extract from sludge castings added to previously uningested sludge (▲), and sludge per se (△) on weight of *E. foetida* (left) and *E. eugeniae* (right).

Figure 2 shows that the toxicity of the castings produced from soil by *E. foetida* can be attributed to a water-insoluble fraction. Addition of aqueous extracts to fresh, uneaten sludge did not result in loss of weight to any of the earthworm species, while sludge extracted with water remained toxic.

Within two weeks of aging, egested soil from *E. foetida* was no longer toxic to *E. eugeniae* and the *Amynthas* spp., though it remained toxic to *E. foetida* for at least four weeks (Fig. 3). After six weeks this material had lost nearly all of its toxicity, since none of the worms died with a mean loss of about 10% of body weight.

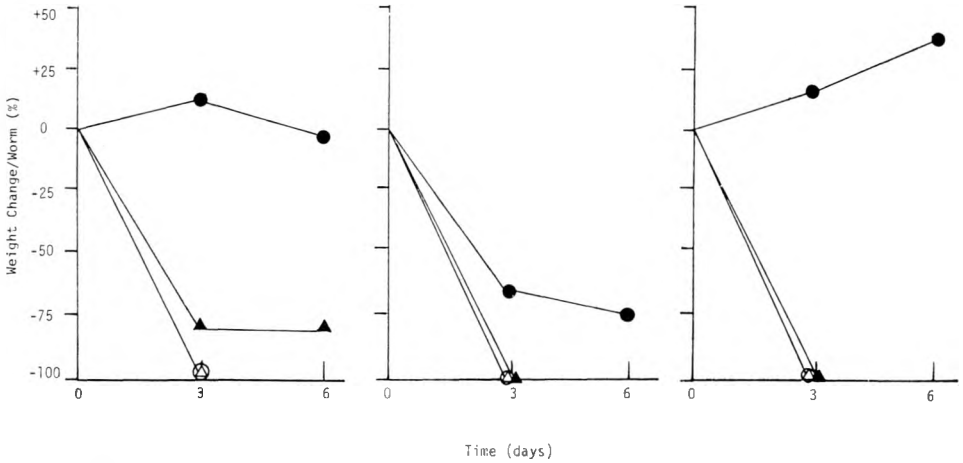

Fig. 7. Effect of sludge (●) and sludge castings, aged 6 weeks, from *E. foetida* (▲), *Amynthas* spp. (△), and *E. eugeniae* (○) on weight of *E. foetida* (left), *Amynthas* spp. (center) and *E. eugeniae* (right).

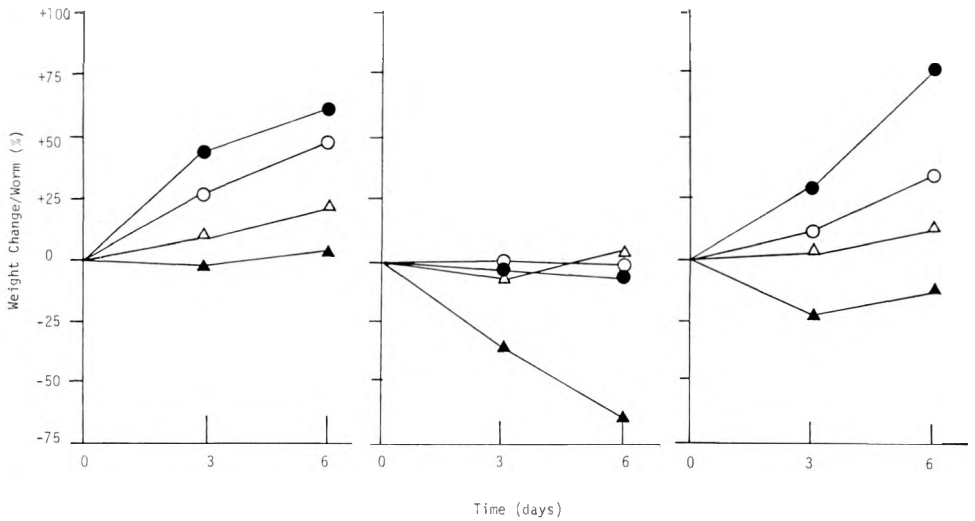

Fig. 8. Effect of soil amended sludge (●), and soil amended sludge castings, aged 6 weeks, from *E. foetida* (▲), *Amynthas* spp. (△), and *E. eugeniae* (○), on weight of *E. foetida* (left), *Amynthas* (center), and *E. eugeniae* (right). In contrast to Fig. 7, the castings had been placed upon soil.

3.2. Sludge castings

Values of *p*H and Eh of the sludge and sludge castings are given in Table 2. The *p*H ranged from 4.7 to 6.8 and Eh from 256 to 451 mv.

3.3. Effect of fresh sludge castings

Figure 4 shows that *E. foetida* and *E. eugeniae* failed to gain weight in a substrate of their own freshly-produced castings, though both species gained weight on confinement to castings of one another and castings of *Amynthas* spp. The *Amynthas* spp. lost 5% of their body weight on fresh sludge, 7% on sludge castings from *E. foetida*, 30% on sludge castings from *E. eugeniae* and 20% on sludge castings produced by themselves. The differences between losses on fresh sludge and sludge castings, the finding of 8% of the wet weight of randomly

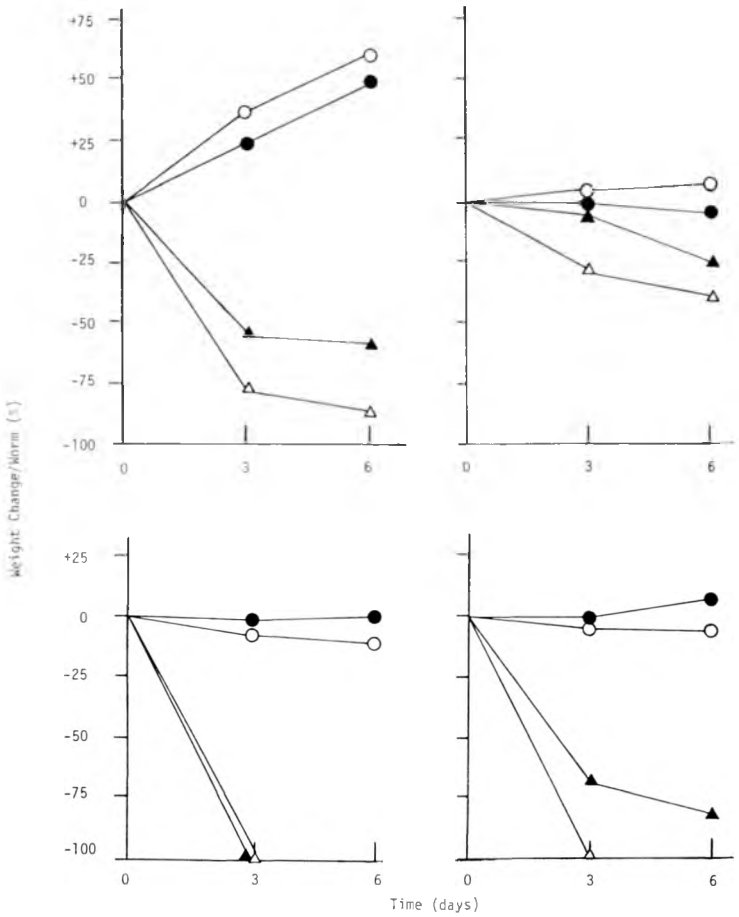

Fig. 9. Intra- and interspecific effects of sludge castings, in relation to aging of castings, on weight of *E. eugeniae* (left) and *E. foetida* (right). *E. eugeniae* feeding on castings from *E. foetida* (upper left) and on its own (lower left). *E. foetida* feeding on castings from *E. eugeniae* (lower right) and on its own (upper right). Age of castings: fresh (●), 1 week old (○), 3 weeks old (▲), 4 weeks old (△).

selected samples of *Amynthas* spp. as gut content, and the ability to measure weight-gain increments on a mineral soil in 6 days suggests that the weight losses in *Amynthas* spp. on sludge castings was due mainly to the unsuitability of sludge or sludge castings as food.

3.4. Effect of soil on toxicity of sludge castings

Placement of fresh sludge castings from *E. eudrilus* upon soil (Fig. 5) resulted in a reduction of the weight-reducing effect seen for sludge castings alone (Fig. 4). Less of a reduction was observed for *E. foetida* (Figs. 4 and 5).

3.5. Causative factor of toxicity in fresh sludge castings

As with the soil castings, the weight losses in *E. eugeniae* and *E. foetida* due to confinement on their own sludge castings can be attributed to a watersoluble fraction (Fig. 6).

3.6. Effect of aging on toxicity of sludge castings

After aging 6 weeks in the absence of soil, the sludge castings produced by each earthworm species were highly toxic to themselves and to one another (Fig. 7). *Amynthas* spp. and *E. eugeniae* feeding on castings from all three species, and *E. foetida* feeding on castings of *E. eugeniae* and *Amynthas* spp. were all dead within three days. *E. foetida*, feeding on their own castings aged 6 weeks, lost 81 % of their weight and displayed 75 % mortality after 6 days. *E. foetida* and *E. eugeniae*, feeding upon uningested sludge aged 6 weeks, maintained or gained weight, while *Amynthas* spp. lost weight (Fig. 7).

3.7. Effect of soil on aged sludge castings

When placed upon soil, sludge castings from *Amynthas* spp. and *E. eugeniae*, previously aged 6 weeks, were innocuous to all the earthworm species, while castings from *E. foetida* aged for the same period were less toxic (Fig. 8) than in the absence of soil (Fig. 7).

3.8. Temporal changes associated with toxicity of sludge castings

In a separate set of experiments, exposure of *E. eugeniae* and *E. foetida* to their own and each other's sludge castings, aged 0, 1, 3 and 4 weeks, revealed the patterns illustrated in Fig. 9. The castings of *E. foetida* were not toxic to *E. eugeniae* one week after production, but after 3 and 4 weeks large losses in weight were seen along with 60 and 80 % mortality following 6 days of exposure. Exposure of *E. foetida* for 6 days to its own castings, aged 3 and 4 weeks, also resulted in increasing weight losses and 25 % mortality. Exposure of *E. foetida* and *E. eugeniae* to castings produced freshly by *E. eugeniae* and the same castings aged 1 week resulted in slight losses of weight in both species. Additional aging to 3 and 4 weeks resulted in 100 % mortality for *E. foetida* and *E. eugeniae* respectively. After aging a total of 9 weeks, the sludge castings from *E. eugeniae* still produced 100 % mortality to *E. foetida* and *E. eugeniae* upon being exposed for 3 days, while the castings from *E. foetida* produced 50 % mortality in 3 days to *E. foetida* and *E. eugeniae*. These results correspond with the results obtained in the previous experiment, where sludge castings were aged for 6 weeks.

3.9. Solubility status of toxicity in aged sludge castings

Extractions of sludge castings aged 6 weeks revealed inconclusive results. Some toxicity can be attributed to the water-insoluble fraction, while in other cases toxicity was lost completely.

3.10. Toxicity of castings in relation to sterility

Exposure of *E. foetida* to autoclaved sludge or sludge castings resulted in 100 % mortality on exposure of the worms to the sterilized material for 2 weeks (Table 3). No mortality occurred in the dishes with non-sterilized sludge, and while mortality was not severe in non-sterilized castings, *E. foetida* suffered a significant loss in weight (Table 3). The oxidation-reduction potentials of these samples revealed appreciable reduction of the chemical constituents in both the sludge and castings caused by autoclaving, though the values obtained (+284, +209) are well within the range of the earthworm's normal habitat.

Exposure of *E. foetida* to irradiated castings did not inflict mortality to the same degree as in non-irradiated castings, but the worms lost a significant percentage of body weight (Table 3). Treatment of sludge with ^{60}Co did not alter the material sufficiently to induce either a toxic effect or a reduction in weight gain upon subsequent exposure of *E. foetida* to treated and control samples (Table 3). In contrast to autoclaving, treatment with ^{60}Co did not reduce the oxidation-reduction potential of the sludge or castings (Table 3).

Table 3. Responses of *E. foetida* to sludges and castings sterilized by autoclaving or irradiation with ^{60}Co

Material	Treatment	Eh (mv)	N	Mortality ($\bar{x} \pm SE$, %)	Change in wt ($\bar{x} \pm SE$, %)
Castings	Steam	+284	4	*100*	—
Castings	Control	+434	4	*19 ± 4.4*	*−25 ± 2.8*[2])
Sludge	Steam	+209	4	*100*	—
Sludge	Control	+371	4	*0*	*+10 ± 4.2*[2])
Castings	Radiation	+281	3	*44 ± 6.5*	*−24 ± 2.0*[3])
Castings	Control	+301	3	*100*	—
Sludge	Radiation	+258	3	*6 ± 5.7*	*+25 ± 1.6*[3,4])
Sludge	Control	+245	3	*11 ± 1.9*	*+32 ± 7.7*[4])

1) The experiment with ^{60}Co was carried out 5 weeks after the experiment on autoclaved samples and are therefore not directly comparable.

2) $P < 0.01$; 3) $P < 0.01$; 4) $P < 0.05$.

4. Discussion

4.1. Eh and *p*H

The toxicity of the soil castings of *E. foetida* correlate somewhat with *p*H. Although this value was lower in the soil castings of all three genera of worms than in the uningested soil, it increased over time for soil castings of *E. foetida*, which also became innocuous to *E. eugeniae* and *Amynthas* spp. and less toxic to *E. foetida* itself. A similar trend for Eh of soil castings was not observed.

Sludge castings also displayed a lower *p*H than the sludge itself, but here, despite an increase in *p*H over 6 weeks in the castings of *E. eugeniae* and *Amynthas* spp., the intraspecific toxicity of these castings increased. Toxicity could be decreased for the castings of these two genera through contact with soil, but not as large a reduction is seen for *E. foetida*, whose sludge castings also failed to exhibit an increase in *p*H as was the case for *E. eugeniae* and *Amynthas* spp. As with soil, no relation was noted between Eh and toxicity.

Autoclaving, but not ^{60}Co irradiation, lowered the redox potential of sludge and sludge castings. Similarly, the combination of high pressure, temperature and moisture caused sludge to become toxic to *E. foetida* whereas the radiation of ^{60}Co did not. It appears that radiation reduced the severity of toxicity of the sludge castings from *E. foetida* to itself, perhaps by destroying pathogenic microorganisms within the castings.

4.2. Probable causative agent of toxicity in castings

The causative agent of toxicity in castings is very likely attributable to a peculiar combination of microorganisms and/or their metabolic excretions. This suggestion is supported by several observations, such as the water-insoluble nature of the toxic agent, and the variable changes in strength of toxicity over time with respect to the source of the castings. Toxicity increased in the castings produced by *E. foetida* and decreased in the castings from *E. eugeniae* and *Amynthas* spp.

Another observation which lends support to the notion that the causative agent lies in certain types of microorganisms has to do with the feeding potential of *E. foetida* on certain populations of microorganisms in various wastes. Freshly voided human excrement (Savage 1977); cow manure (Hungate et al. 1964), and anaerobic sludge digests derived from domestic sewage (Crowther & Harkness 1975) contain a microbial population which is initially virtually devoid of protozoa. After 2 days at 25 °C, large numbers of these Protista appear in fresh cattle manure exposed to air, and about 1 day still later *E. foetida* begins to feed upon this waste (unpublished observations). Similarly, *E. foetida* exhibits little or

no tendency to feed upon fresh human excrement or anaerobic sludge, and usually dies within several hours to days when forced to remain upon these materials in well-aerated, closed containers. When the microbial composition of these wastes is altered by exposure to activated sludge, however, the resultant microbial community (CURDS 1975; PIKE 1975) is highly supportive of weight gain by *E. foetida* (NEUHAUSER 1978). This conversion process may partly explain the ameliorative action observed for soil in this study. Also, possibly the destruction of certain microbes by ^{60}Co provided the ameliorative effect shown in Table 3.

Additional support of the suggestion that toxicity resides basically in the microbial community in the castings can be drawn from observations on the peritrophic membrane which *E. foetida* produces (GANSEN 1963). The presence of this membrane around freshly voided sludge castings was deduced microscopically by one of us (RH) at 100× and 540×, by pressing upon a cover slip and noting the restraining effect the membrane imposed upon the microbes and other particulates within the casting (unpublished observation).

4.3. Waste management

A potential exists for using earthworms in waste management, but from the results presented here, earthworms cannot be exposed to high concentrations of sludge castings for long periods of time. Soil, possibly an unknown component thereof, or periodic additions of activated sludge, with a microbial population and physical and chemical conditions beneficial to *E. foetida* and *E. eugeniae* may be employed to facilitate the waste conversion process, but additional experiments are needed to establish the procedure on a scientific foundation. A better understanding of coprophagic relations among the dominant earthworm species in any ecosystem, coupled with the introduction of *E. foetida* and/or *E. eugeniae* where edaphic factors and climate will allow, should permit preclusion of problems introduced by spreading amounts of slurry on the landscape (CURRY 1975) and should allow better use of soil for coping with wastes.

5. Summary

Castings produced from a mineral soil by *E. foetida* were toxic to itself, *E. eugeniae* and two *Amynthα* spp., while castings produced from the soil by *E. eugeniae* and *Amynthas* spp. allowed a gain or maintenance of weight to all three earthworms. Toxicity was due to a water-insoluble fraction of the castings. Interspecific toxicity disappeared after two weeks of aging the soil castings at 25 °C, at which time the toxicity of soil castings to *E. foetida* was reduced.

Castings produced from activated sludge by *E. foetida* and *E. eugeniae* and fed upon by those species resulted in weight losses to themselves but not to each other. Toxicity was due to a water insoluble component. Placement of these sludge castings on soil resulted in a reduction of the weight-reducing effect. After aging for six weeks at 25 °C in the absence of soil, the sludge castings from each earthworm species were toxic to themselves and to each other. Placement of these castings on soil eliminated the toxicity of sludge castings produced by *E. eugeniae* and *Amynthas* spp. to all three earthworms and reduced the toxicity of *E. foetida* castings.

Reasons are advanced to support a suggestion that intraspecific and interspecific toxicity of sludge castings can be attributed to certain microbial populations within the castings, and implications of these results in waste management practices are discussed.

Koprophage Beziehungen zwischen den Regenwurmarten *Eisenia foetida*, *Eudrilus eugeniae* und *Amynthα* spp.

Losung von *Eisenia foetida* aus Mineralboden war für diese Art sowie für *Eudrilus eugeniae* und zwei *Amynthas* spp. toxisch, während als Nahrung angebotene Losung aus dem gleichen Bodenmaterial, die von den beiden letztgenannten Arten produziert wurde, Würmern aller drei Arten einen Gewichtszuwachs bzw. die Erhaltung des Körpergewichts ermöglichte. Die Toxizität wurde auf wasserunlösliche Verbindungen in der Losung zurückgeführt. Die interspezifische Toxizität verschwand nach 2wöchiger Alterung der Losung bei 25 °C, in der Zeit, in der die Toxizität von Mineralboden-Losung gegenüber *E. foetida* vermindert wurde.

Losung die aus aktiviertem Schlamm von *E. foetida* und *E. eugeniae* ausgeschieden wurde und die Würmern der gleichen Arten als Nahrung geboten wurde, führte, jeweils bei der gleichen Art zu Gewichtsverlusten, nicht aber bei der jeweils anderen Art. Auch hier wurde die Toxizität von einer wasserunlöslichen Komponente hervorgerufen. Die Plazierung solcher Schlamm-Losung auf die Bodenoberfläche führte zu einer Verringerung ihrer gewichtsvermindernden Wirkung. Nach einer

6wöchigen Alterung bei 25 °C und Abwesenheit von Bodenmaterial war die Losung der genannten Arten [dagegen] toxisch für sie selbst und auch für die jeweils andere Art. Die Plazierung dieser Losungen auf die Bodenoberfläche eliminierte die Toxizität der Schlamm-Losung von *E. eugeniae* und *Amynthas* spp. gegenüber allen drei genannten Arten und sie verminderte auch die Toxizität von *E.-foetida*-Losung.

Motivationen wurden gefunden, um die Vermutung zu stützen, daß die intraspezifische und die interspezifische Toxicität von Schlamm-Losung auf gewisse Mikroben-Populationen in der Losung zurückgeführt werden kann und Möglichkeiten der Anwendung dieser Resultate in die Praxis der Abfallwirtschaft werden diskutiert.

6. References

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